GROUND DERRIS ROOT AS A MOSQUITO LARVICIDE.

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Introduction.

The first reference to ground derris root as a mosquito larvicide, as far as can be ascertained, occurs in a private communication by Durham in 1902, mentioned by Gimlette (1923), in which he stated that derris powder, 1 in 40,000 parts and greater concentrations, was toxic to *Culex* sp.

De Ong and White (1924) stated that derris powder sprinkled on the surface of the water killed 90% of mosquito larvae in 2–4 hours.

Castillo (1926) allowed the coarser particles of a suspension of derris powder to fall to the bottom of a jar, separated mosquito larvae from this sediment by cheese-cloth and found that the maximum toxicity was obtained by using 3 grams of powder per 1,000 c.c. of water. Both weaker and stronger mixtures were less effective. This result is very difficult to understand, since the results reported in the present paper show a definite increase in toxicity with increased concentrations of derris.

Twinn (1927) reported that in laboratory experiments, derris powder dusted on water at the rate of 3 lb. per acre gave a complete kill of mosquito larvae in 7 hours, and pupae in 24 hours. Field experiments, using 1 part of derris to 4 parts of French chalk at the rate of 1½ lb. of derris per acre, gave a complete kill of *Aëdes vexans* Mg. larvae within 60 hours.

Gibson (1928) stated that in laboratory experiments, derris at the rate of 15 lb. per acre gave 100% kill of *Aëdes* sp. larvae and pupae in 22 hours and 5 days, respectively. Field experiments at the rate of 30 lb. per acre gave 100% kill of *Culex pipiens* Linn. larvae in 72 hours. The same author (1929) in further field experiments stated that derris at the rate of 5 lb. per acre was definitely unsatisfactory. It should be noted that the two previous authors took no account of the volume of the water, but merely estimated the surface area.

Wille *et al.* (1937) stated that cubé root containing 5% rotenone had no effect on the larvae of *Anopheles pseudopunctipennis* Theo. when used at a concentration at which it killed fish in 30 minutes.

Roman and Nétien (1939) stated that derris powder dusted onto the surface of the water, at the rate of 2 milligrams per litre, killed larvae of *Anopheles* sp. in 3 days, but that a concentration of 10 milligrams per litre was ineffective when the powder was wetted and submerged. These authors also stated that the powder was only slightly toxic to the larvae of *Culex* sp.

Mosquito larvae have been widely used in comparative toxicity tests for rotenone as compared with other insecticides, but in all such experiments extracts containing rotenone and other derris products were used and not derris powder.

The experiments reported in this paper were undertaken to determine whether derris could be used effectively under certain conditions as a control for the larvae of Australian species of mosquitoes.

Material and Methods.

The method of breeding the larvae for this work was similar to that used by Woodhill (1936). Except where otherwise stated, early 3rd instar larvae were used in each experiment.

The composition of the derris powder used was:

Total extractible matter	19·8%
Rotenone	3·8%

The weighed amounts of derris powder were first thoroughly wetted and mixed with 1,000 c.c. of tap-water. This was then divided as evenly as possible between 4 flat-bottomed dishes of approximately 300 c.c. capacity. To each dish was then added 0·04 gm. of well-wetted fish food and yeast (Woodhill, 1936). By means of a small pipette, 25 larvae were then transferred from the stock lot to each dish, making a total of 100 larvae used for each concentration of material.

The temperature was maintained at 80°F. and the humidity varied between 70–80%. The maximum time allowed for a kill was 48 hours, but the times given for the mortality in each table of concentrations do not represent the exact length of time taken for that percentage mortality.

Experiments and Results.

Experiment 1.—To determine the toxicity of different concentrations of ground derris root in aqueous suspension, and possibly solution, plus a standard amount of fish food and yeast, to 3rd instar larvae of *Culex* (*Culex*) *fatigans* Wied. The results, as set out in Table 1, show that the minimum concentration of ground derris root necessary to give 100% mortality within 48 hours was 0·01 gm./1,000 c.c.

Table 1.

Concentration.	Time, Hours.	Mortality, %
(*a*) 0·2 gm./1,000 c.c.	17	100
(*b*) 0·2 gm./1,000 c.c.	4	100
Control	48	0
(*a*) 0·1 gm./1,000 c.c.	17	100
(*b*) 0·1 gm./1,000 c.c.	4	100
Control	48	0
(*a*) 0·05 gm./1,000 c.c.	15	100
Control	48	1
(*b*) 0·05 gm./1,000 c.c.	10	100
Control	48	0
(*a*) 0·04 gm./1,000 c.c.	7	100
Control	48	1
(*b*) 0·04 gm./1,000 c.c.	10	100
(*c*) 0·04 gm./1,000 c.c.	4	100
Control	48	0
(*a*) 0·02 gm./1,000 c.c.	21	100
Control	48	1
(*b*) 0·02 gm./1,000 c.c.	8	100
Control	48	0
(*a*) 0·01 gm./1,000 c.c.	48	99
(*b*) 0·01 gm./1,000 c.c.	39	100
(*c*) 0·01 gm./1,000 c.c.	30	100
(*d*) 0·01 gm./1,000 c.c.	25	100
Control	48	0
0·009 gm./1,000 c.c.	48	89
Control	48	0
0·007 gm./1,000 c.c.	48	82
Control	48	0
0·006 gm./1,000 c.c.	48	80
Control	48	0
(*a*) 0·005 gm./1,000 c.c.	48	41
(*b*) 0·005 gm./1,000 c.c.	48	70
Control	48	0

There was 100% mortality in concentration (*a*) 0·01 gm./1,000 c.c. after 56 hours.

Experiment 2.—To determine the toxicity of different concentrations of ground derris root in aqueous suspension, and possibly solution, without the addition of any fish food and yeast, to larvae of *Culex* (*Culex*) *fatigans* Wied. The results, which are set out in Table 2, indicate that a certain ratio exists between the amount of food present

Table 2.

Concentration.	Time, Hours.	Mortality, %
0·01 gm./1,000 c.c.	18	100
Control (no food)	48	0
0·008 gm./1,000 c.c.	18	100
Control (no food)	48	0

and the percentage mortality and also between the amount of food present and the time taken for 100% mortality (see Phillips and Swingle, 1940, and compare these results with those in Table 1).

Experiment 3.—To determine the toxicity of concentrations of ground derris root in aqueous suspension, and possibly solution, plus a standard amount of fish food and yeast, to 1st, 2nd and 4th instar larvae of *Culex* (*Culex*) *fatigans* Wied. The results, which are set out in Table 3, show that derris is toxic to all the instars tested, and the minimum concentration for 100% mortality within 48 hours rises with each succeeding instar.

TABLE 3.

Concentration.	Instar.	Time, Hours.	Mortality, %
0·02 gm./1,000 c.c.	4th	10	100
Control	,,	48	0
0·008 gm./1,000 c.c.	2nd	20	100
Control	,,	48	0
0·006 gm./1,000 c.c.	1st	7	100
Control	,,	48	0

Experiment 4.—Three weights of derris powder, 0·05 gm., 0·02 gm. and 0·01 gm., were each thoroughly wetted and mixed with 1,000 c.c. water by mixing in a bottle on a revolving machine for 24 hours. Each mixture was then filtered through a Whatman No. 1 chemical filter-paper in a suction filter pump. The toxicity of the filtrates of these three concentrations of ground derris root, plus a standard amount of fish food and yeast, to 3rd instar larvae of *Culex* (*Culex*) *fatigans* Wied., was then determined. The results, which are given in Table 4, are interesting in so far as they show that the filtrate is almost as toxic as the straight-out aqueous suspension, and possibly solution, of ground derris root.

TABLE 4.

Concentration.	Time, Hours.	Mortality, %
0·05 gm./1,000 c.c.	15	100
Control	48	0
(*a*) 0·02 gm./1,000 c.c. ..	48	97
(*b*) 0·02 gm./1,000 c.c. ..	20	100
Control	48	0
0·01 gm./1,000 c.c.	48	91
Control	48	0

Experiment 5.—To determine the effect upon the toxic principle of ground derris root in aqueous suspension, and possibly solution, after standing for a time exposed to light, but not directly to the sun, to 3rd instar larvae of *Culex* (*Culex*) *fatigans* Wied. The same standard amount of fish food and yeast was used in each experiment and the concentration was allowed to stand for the required number of days before the larvae were introduced. Each concentration was examined at 24-, 36- and 48-hourly intervals, so that the times mentioned in Table 5 do not represent the exact time taken for the mortality shown. The results, as set out in Table 5, indicate that the toxicity of derris is slightly reduced by standing for 8 days or more.

Table 5.

Time of Standing, Days.	Concentration.	Time, Hours.	Mortality, %
2	0·02 gm./1,000 c.c.	24	100
	Control	48	0
4	0·02 gm./1,000 c.c.	24	100
	Control	48	0
8	0·02 gm./1,000 c.c.	36	100
	Control	48	0
9	0·02 gm./1,000 c.c.	48	96
	Control	48	0

Experiment 6.—To determine the toxicity of different concentrations of ground derris root in aqueous suspension, and possibly solution, plus a standard amount of fish food and yeast, to early 3rd instar larvae of the salt-water mosquito, *Aëdes* (*Pseudoskusea*) *concolor* Tayl., to see if salt-water adversely affected the toxicity of derris under such conditions. One hundred larvae used for each concentration, the salt-water being sea-water with a salinity of 35·5. From Table 6 it is evident that ground derris root gave precisely the same kill with larvae of *A.* (*Pseudoskusea*) *concolor* Tayl. in sea-water as with larvae of *Culex* (*Culex*) *fatigans* Wied. in freshwater.

Table 6.

Concentration.	Time, Hours.	Mortality, %
0·02 gm./1,000 c.c.	22	100
Control	48	0
0·01 gm./1,000 c.c.	22	100
Control	48	0

Experiment 7.—A concentration of 0·04 gm./1,000 c.c. was used in a rock pool at Harbord, Sydney, containing 1st, 2nd, 3rd and 4th instar larvae and pupae of *A.* (*Pseudoskusea*) *concolor* Tayl., and three rock pools nearby were used as controls. The ground derris root was dispersed evenly through the pool by wetting the required amount of material in a bottle with sea-water from the pool and then distributing it evenly over the whole surface of the pool from whence it sank and dispersed through the water.

There was some organic detritus in the pool. The weather was warm and sunny during the experimental period of 48 hours.

At the end of this period the pool was examined. One hundred per cent. mortality of the larvae had resulted and most of the pupae were dead. There was no mortality in the control pools.

Discussion.

It will be seen from these experiments that derris powder is an efficient larvicide against *Culex* (*Culex*) *fatigans* Wied. and *Aëdes* (*Pseudoskusea*) *concolor* Tayl. and would possibly be just as effective against other species of *Culex* and *Aëdes*.

It should be noted, however, that the quantity of derris powder used must be calculated on the volume of water and not on the surface area.

Although 0·01 gm./1,000 c.c. of derris gave a complete kill of larvae in the laboratory, it would be advisable to use 0·02 gm./1,000 c.c. of derris in the field.

Preliminary field experiments only have been carried out and further work is desirable. It should be realized, however, that derris will destroy most other insects, crustaceans and fish in the water and that, in very strong concentrations, it may be fatal

to man and animals (Shepard, 1940). Although the chance of poisoning occurring as a result of treating water at the rate suggested is highly improbable, its use in drinking-water tanks is not recommended, since other methods of control can be conveniently used. According to Martin and Tattersfield (1939), rotenone rapidly loses its toxicity on exposure to sunlight, but no details are available as to its behaviour in this regard under Australian conditions.

It is not contended that derris is likely to supersede oil or Paris green, but in certain circumstances it may be useful as an alternative method for treating particular types of breeding places. Its chief value lies in the certainty and rapidity of its action and the ease with which it may be transported and applied. There is considerable evidence (Twinn, 1927; Gibson, 1928) that it is also toxic to pupae, and preliminary work by the authors tended to confirm this, but the experiments were not extensive enough to enable definite conclusions to be drawn. Since, as indicated later, it has little or no harmful effect on bacteria, it could be used in sewage treatment works.

Mr. J. M. Vincent, Lecturer in Bacteriology, School of Agriculture, University of Sydney, kindly carried out some tests of derris in relation to bacteria, and supplied the following note: "Derris root was tested at two final concentrations of 1/10,000 and 1/20,000, against three organisms likely to be representative of the kind found in a septic tank, viz., *Proteus vulgaris*, *Bacterium coli* and *Bacillus mycoides*. However, derris controls, without any organisms added, showed such abundant growth as to mask completely the growth of the pure cultures which were added. For this reason it seems most unlikely that the suspension in the concentrations tested would have an inhibiting effect on bacteria. Derris added to MacConkey's bile salt medium (which is rather selective for organisms that commonly inhabit the intestinal tract), still gives abundant growth and plenty of gas production. This would indicate further that some of the organisms introduced with the derris and growing vigorously in its presence are quite likely to be representative of the type likely to be developing in a septic tank. Additionally, it might be noted that it was just possible to detect an effect (detectable in spite of the vigorous spontaneous growth) due to *Bact. coli* added in pure culture to MacConkey's containing both concentrations of derris."

Summary.

1. The minimum concentration of ground derris root which causes 100% mortality of early 3rd instar larvae of *Culex* (*Culex*) *fatigans* Wied. in 48 hours is 0·01 gm./1,000 c.c.

2. When no food material is available the minimum concentration necessary for 100% mortality is lowered, as is also the time taken for this mortality.

3. Ground derris root is toxic to all instars of larvae of *Culex* (*Culex*) *fatigans* Wied.; the minimum concentration necessary for 100% mortality rising with each succeeding instar.

4. The filtrate obtained by filtering a concentration of ground derris root, well shaken in water for 24 hours through a chemical filter-paper, Whatman No. 1, gives 100% mortality in 20 hours in concentrations as low as 0·02 gm./1,000 c.c.

5. A concentration of 0·02 gm./1,000 c.c. has been found to give 100% mortality in 48 hours after standing for 8 days, but not exposed to direct sunlight.

6. Ground derris root is just as toxic in sea-water to larvae of *Aëdes* (*Pseudoskusea*) *concolor* Tayl. as it is to larvae of *Culex* (*Culex*) *fatigans* Wied. in freshwater.

7. In a field test, using 0·04 gm./1,000 c.c. of ground derris root, in sea-water pools 100% mortality of the larvae and a considerable mortality of pupae of *Aëdes* (*Pseudoskusea*) *concolor* Tayl. were obtained in 48 hours.

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